AD_____

Award Number: DAMD17-00-1-0605

TITLE: Rapid Screen for Tumor Suppressor Genes on Chromosome 19p13

PRINCIPAL INVESTIGATOR: Steffi Oesterreich, Ph.D.

CONTRACTING ORGANIZATION: Baylor College of Medicine
Houston, Texas 77030

REPORT DATE: September 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2002	Final (1 Sep 00 - 31 Aug 02)

4. TITLE AND SUBTITLE
Rapid Screen for Tumor Suppressor Genes on Chromosome 19p13

5. FUNDING NUMBERS
DAMD17-00-1-0605

6. AUTHOR(S):
Steffi Oesterreich, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Baylor College of Medicine
Houston, Texas 77030
E-Mail:
steffio@breastcenter.tmc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

.We have recently found that a region on chromosome 19p13 displays an unusually high rate of allelic imbalance (LOH). In 78% of microdissected breast cancer specimens one allele copy was lost. The LOH rate at this locus is highest at the polymorphic marker D19S216, and is lower at nearby up- and downstream markers. Such loss is a hallmark of tumor suppressor genes. Although we are currently investigating one promising candidate for a tumor suppressor gene at this locus (named HET/SAF-B), we expect that this "hot spot" LOH region harbors more than one critical gene. This sort of clustering has been seen in other regions of high LOH; for example, a very small region on chromosome 13q (<25 cM) harbors a series of interesting genes including BRCA2, Brush1, FKHR, and Rb. We propose to use a new method to rapidly screen the 19p13 region for other candidates. ESTs from the region of high LOH (19p13.2-3) will be arrayed onto filters, and candidates will be identified based on decreases in DNA copy number (loss of one allele, or homozygous loss) in breast tumors. In a subsequent study the same arrays will be used to study gene expression using mRNA. Finally, the identified candidate genes will either be verified or eliminated as tumor suppressor genes by mutation analysis in breast tumors, and by functional assays. Depending on how many potential candidates we will identify, we will at first focus on genes for which DNA copy number as well as RNA expression is lost.

14. SUBJECT TERMS	15. NUMBER OF PAGES
array, LOH, chromosome 19p13	5
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Cover.....1

SF 298.....2

Table of Contents.....3

Introduction.....4

Body.....4

Key Research Accomplishments.....5

Reportable Outcomes.....5

Conclusions.....5

References.....5

Appendices.....

Introduction

We have previously reported that a region on chromosome 19p13 displays extremely high rates of loss of heterozygozity (LOH) (ref). LOH is a hallmark for the existence of tumor suppressor gene. We proposed to identify potential candidates through the use of cDNA microarrays. Therefore we would spot cDNAs from genes mapping to the high LOH area onto chips, and hybridize them with genomic DNA from breast tumors. The same arrays could be used in a subsequent study to measure RNA expression of genes from the high LOH area. In studies performed outside this proposal, we would analyze potential candidates through functional studies.

Body

A first step to perform the proposed research was to generate a conclusive list of ESTs mapped to the region of LOH on chromosome 19p13. During the last year a lot of progress was made through the Human Genome Project (www.ncbi.nlm.nih.gov) and through the Lawrence Livermore Lab in sequencing chromosome 19. Using the available sequence information we created a list of 120 unique cDNAs which we were interested in spotting onto arrays. In addition we listed genes which are known to be lost or amplified in breast cancer, which would serve as appropriate controls. The majority of those clones were purchased from our Microarray Facility here at Baylor College of Medicine (they originally purchased these clones from Research Genetics). The bacterial clones were grown up, and DNA was amplified using M13 forward and reverse primers. The clones which were not available from our Core Facility were purchased from Research Genetics.

As mentioned previously, a number of clones gave more than one PCR product which we could not eliminate through optimization of our PCR conditions. Those clones were restreaked, and the analysis of more colonies revealed that a number of clones contained more than one clone. Also, some clones did not result in any PCR products. And finally, upon sequence analysis, we realized that yet other clones did not contain the appropriate cDNA. Thus we have had various problems with approximately 30% of the cDNA clones. These problems resulted in significant time loss due to additional work needed to obtain all necessary PCR products for the array. Similar problems were recently published as a "News Feature" in Nature (2). The authors describe that sequence analysis of 1,289 IMAGE clones from Research Genetics revealed that only 62% of the stocks definitely represented a pure sample of the correct clone.

The DNA concentration was "normalized", i.e. all samples were diluted to the same concentration of 100ng/µl, and than the DNA samples (total 288) were spotted onto 30 custom arrays. These custom arrays were hybridized with fluorescent labeled cDNA generated from 10 µg total RNA each sample. For this preliminary work we used RNA from normal and breast cancer cell lines. This work was performed in the Baylor College of Medicine Microarray Core Facility, headed by Dr. Lisa White. The data are currently being analyzed. Should the data be good, as we expect, the next step will be to hybridize the arrays with tumor RNA, and subsequently with DNA from LOH-positive and negative tumor.

Key research accomplishments:
-Generated conclusive list of ESTs covering the high LOH, and controls for LOH and amplification in breast cancer, and PCR amplified approx 200 cDNAs for the custom array after overcoming problems with IMAGE clones
-30 custom arrays were printed at the Baylor College of Medicine Core Facility, and have been hybridized with normal and breast cancer cell line RNA

Reportable Outcomes
Poster at the DOD Meeting "Era of Hope", September 25-29, 2002 , in Orlando, Florida.

Conclusions

Troubleshooting resulted in the realization that a significant number of IMAGE clones are problematic at least. Despite these issues which are obviously causing problems for the whole scientific community we were able to generate a set of cDNAs covering the LOH area of which most of them are correct, i.e. contain the correct sequenced gene of interest. Due to those uncontrollable circumstances it took us longer than expected to generate the complete set of PCR products for the chromosome 19p13 cDNA array.

However we now have the custom arrays, and they have recently been hybridized with RNA from normal and breast cancer cell lines. The data are being analyzed, and we expect to detect low expression of genes near to the SAFB locus.

References

1. Oesterreich S, Allred DC, Mohsin SK, Zhang Q, Wong H, Lee AV, Osborne CK, O'Connell P. High rates of loss of heterozygosity on chromosome 19p13 in human breast cancer. Br J Cancer Feb; 84(4):493-498 2001.
2. Jonathan Knight. When the chips are down. News Feature in Nature; 410, 860-861, 2001.